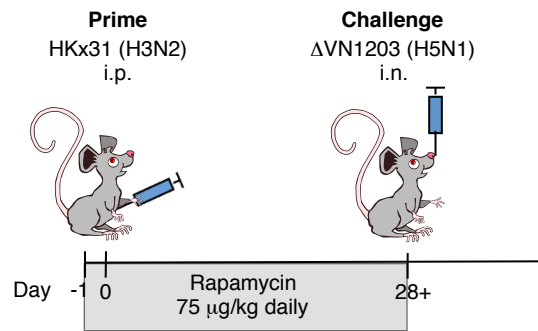
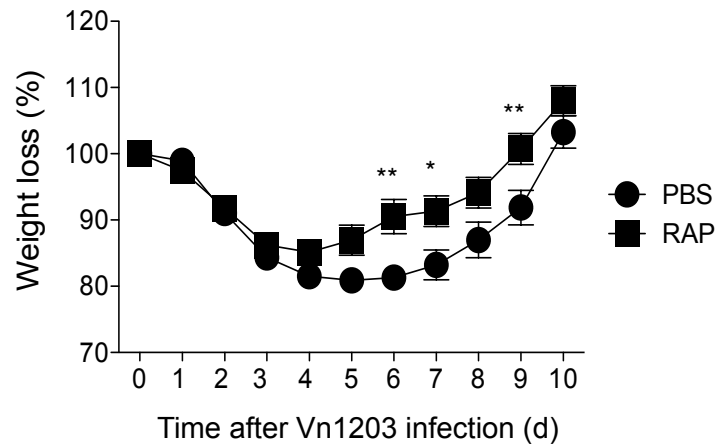


mTOR modulates the antibody response to provide cross-protective immunity to lethal influenza infections.

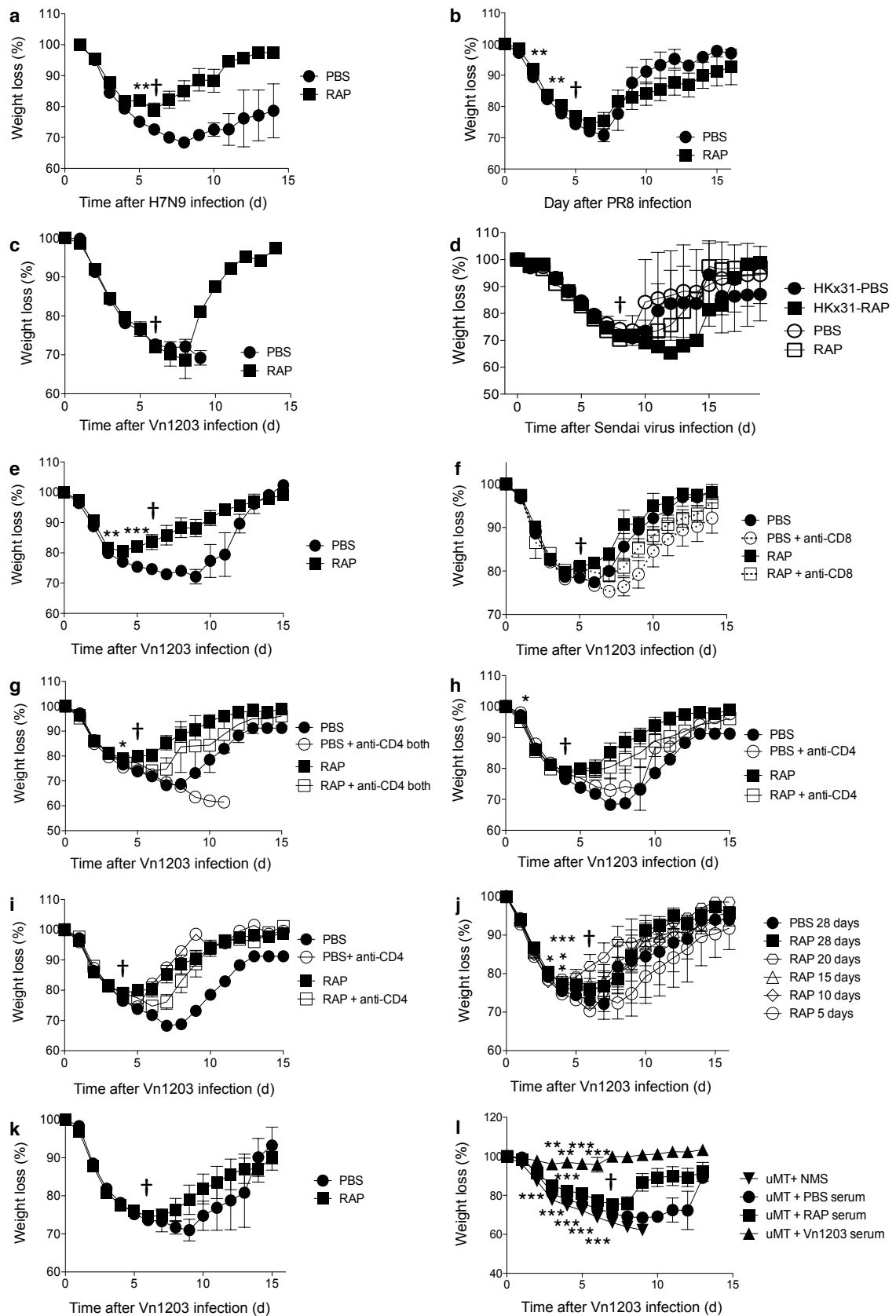
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Supplemental Figure 1 Experimental design. C57BL/6 mice received 75 $\mu\text{g/kg}$ rapamycin or PBS, i.p. beginning 1 day prior to primary infection with 10^8 EID₅₀ of the H3N2 HKx31 i.p. Rapamycin treatment continued daily for 28 days. On day 28, mice were challenged with 4.5×10^5 EID₅₀ of the H5N1 ΔVn1203 i.n.

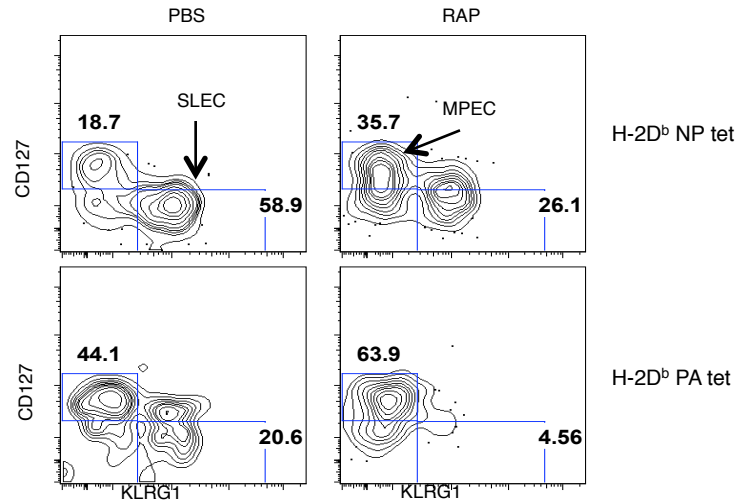


Supplemental Figure 2 Rapamycin enhances protection against a lethal H5N1 infection following i.n. infection with LAIV. C57BL/6 mice received 75 $\mu\text{g/kg}$ rapamycin or PBS, i.p. beginning 1 day prior to and daily for 28 days after primary infection with 10^6 TCID₅₀ HKx31_{ts}. On day 28, mice were challenged with 4.5×10^5 EID₅₀ of ΔVn1203 i.n. and monitored for weight loss (* $P < 0.05$ and ** $P < 0.01$, Two-way ANOVA with Bonferroni multiple comparison, $n = 16$ per group). Data are representative of 2 independent experiments

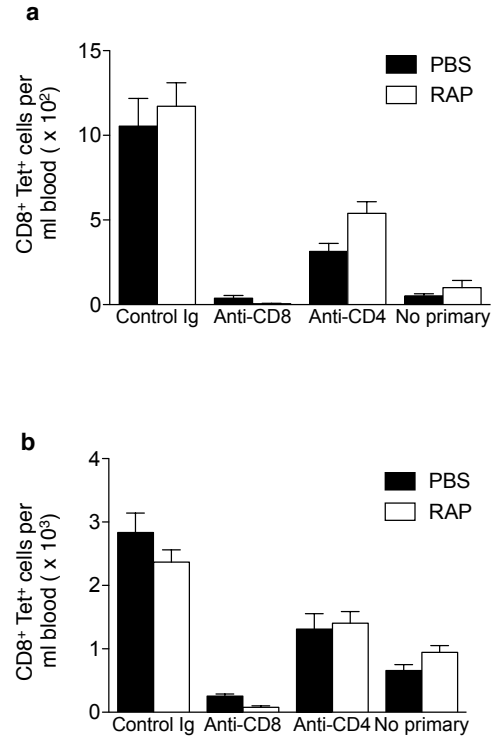


Supplemental Figure 3 Weight loss data following challenge experiments.

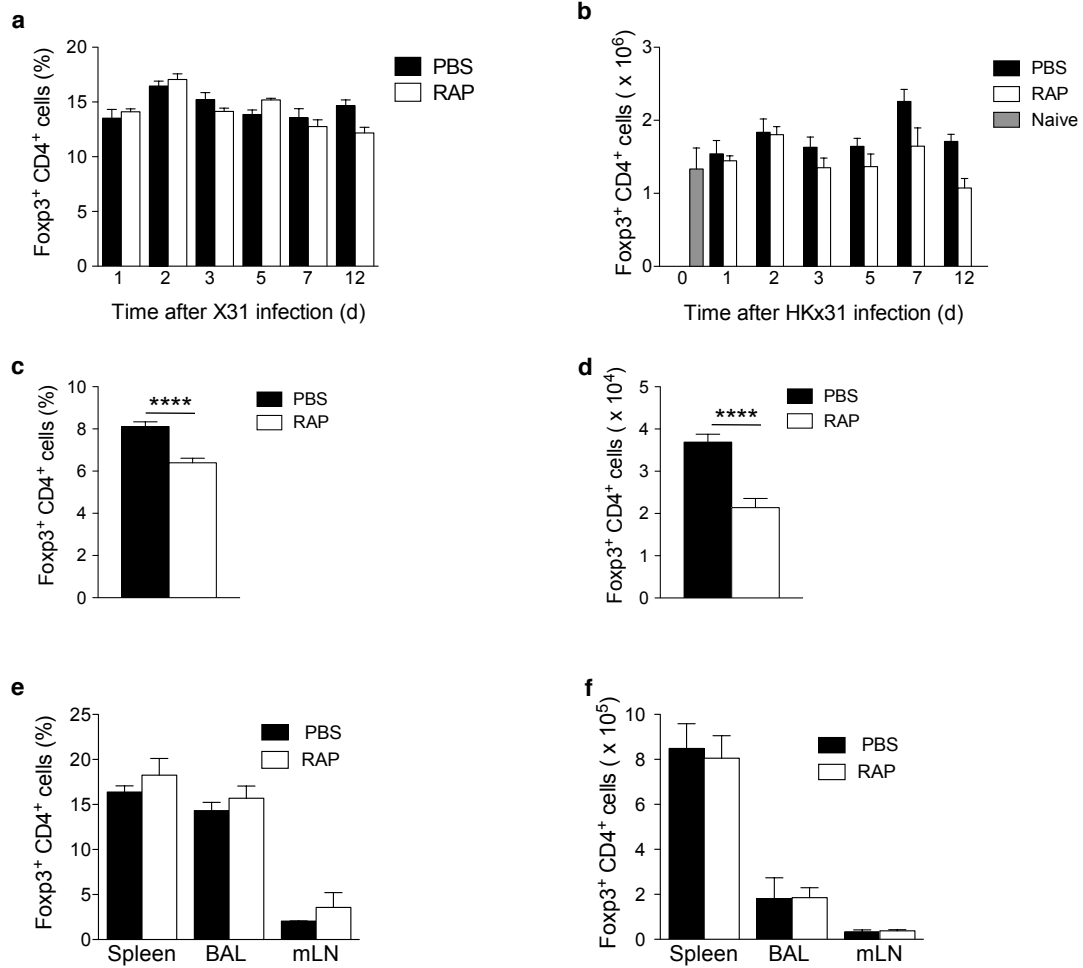
Supplemental Figure 3 Weight loss data following challenge experiments. Statistical analysis was performed using Two-way ANOVA with Bonferroni multiple comparison until the first mouse death occurred (+). Differences were non-significant unless stated otherwise with $*P < 0.05$, $**P < 0.01$, or $***P < 0.001$. All data are representative of at least two independent experiments. Mice were infected with 10^8 EID₅₀ of H3N2 HKx31 virus i.p., treated with rapamycin daily and challenged i.n. on day 28 with either (a) 4.5×10^5 EID₅₀ of TCID₅₀ of A/Anhui/1/2013 ($n = 16$ per group) or (b) 4.5×10^5 EID₅₀ of PR8. ($n = 18$ per group). (c) Mice were treated with rapamycin or PBS for 28 days, infected with Δ Vn1203 i.n., $n = 9$ per group. (d) Mice were infected with HKx31 virus i.p., treated with rapamycin or PBS daily for 28 days and infected with 2×10^4 pfu of Sendai virus i.n. Control groups received PBS or rapamycin daily with no HKx31 primary infection, $n = 8$ per group. (e) Mice were infected with Δ CD8-HKx31 virus i.p., treated daily with rapamycin or PBS, and challenged with Δ Vn1203. ($n = 9$ per group). (f) CD8⁺ T cells were depleted, and mice were infected with HKx31 virus, treated daily with rapamycin or PBS daily, and challenged with Δ Vn1203, $n \geq 23$ per group. (g-i) Mice were infected with HKx31, received rapamycin or PBS daily, and were challenged with Δ Vn1203. CD4⁺ T cells were depleted during either the (g) primary infection and prior to the secondary infection, ($P < 0.05$ for PBS and anti-CD4 versus RAP, $n \geq 9$) or (h) during the primary infection ($P < 0.05$ for PBS and anti-CD4 versus RAP and anti-CD4, $n \geq 6$ per group) or (i) prior to the secondary infection, $n \geq 6$ per group. (j) Mice were infected with HKx31 virus, received rapamycin or PBS daily for the indicated days, and were challenged with Δ Vn1203 28 days following the primary HKx31 infection, ($P < 0.05$ for RAP 15 versus RAP 5 at d5, and RAP 20 versus RAP 5 at d4, and PBS 28 versus RAP 20 at d5; $P < 0.001$ for RAP 20 versus RAP 5 at d5, $n \geq 8$ per group). (k) Mice were infected with HKx31 and received rapamycin or PBS daily for 28 days. Six weeks later, the mice were challenged with Δ Vn1203. (l) One day prior to infection with Δ Vn1203, μ MT mice received 450 μ l of serum i.p. from HKx31-infected mice treated with PBS or rapamycin. Control mice received normal mouse serum or serum from Δ Vn1203-infected mice. ($P < 0.05$ for μ MT + RAP serum versus μ MT + Vn1203 serum, at d2 and μ MT + PBS serum versus μ MT + Vn1203 serum at d2; $P < 0.01$ for μ MT + NMS versus μ MT + RAP serum at d3 and d4; $P < 0.001$ for μ MT + NMS versus μ MT + RAP serum at d5 and d6, μ MT + RAP serum versus μ MT + Vn1203 serum at d5 and d6, μ MT + NMS versus μ MT + Vn1203 at d3, d4, d5, and d6, μ MT + PBS serum versus μ MT + Vn1203 serum at d3, d4, d5, and d6, $n \geq 8$ per group).



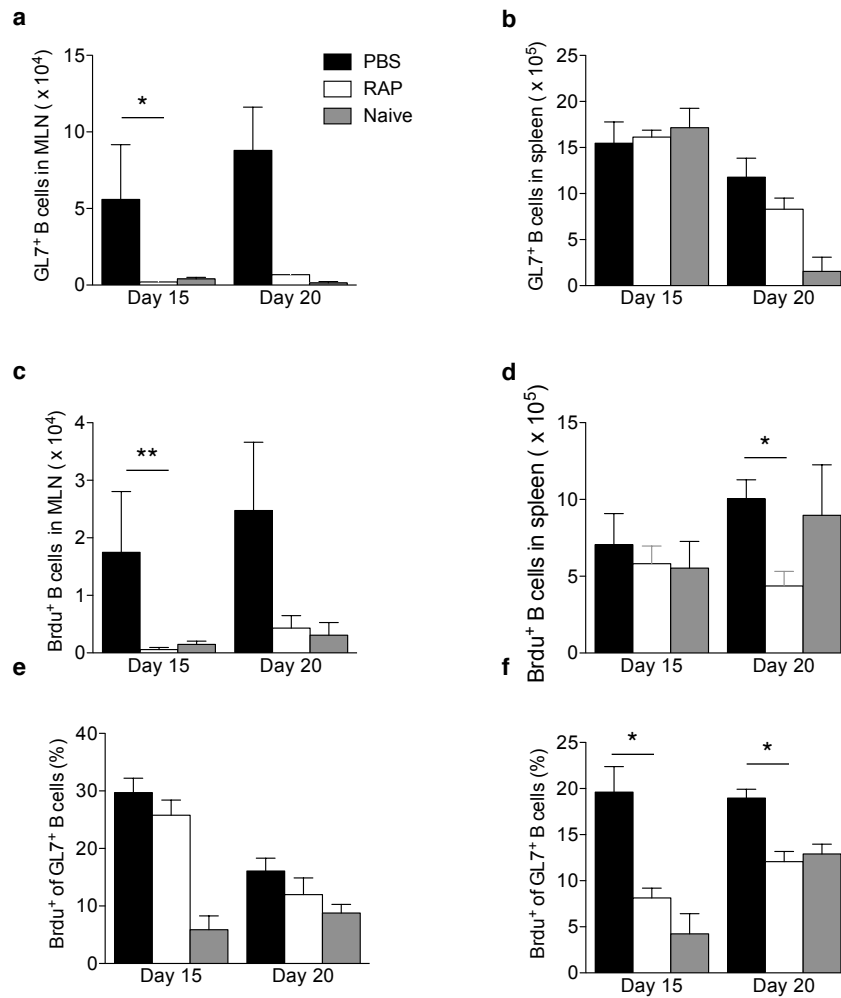
Supplemental Figure 4 Rapamycin increases the number of CD8⁺ T cells with a memory precursor phenotype. Mice were infected with HKx31 and received rapamycin or PBS daily as described in **Fig. 1**. On day 27, a sample of blood from each mouse was analyzed for H-2D^b NP₃₆₆ and H-2D^b PA₂₄₄ tetramer binding, CD8 α , KLRG1 and CD127 expression. Each density plot shows CD127 versus KLRG1 on electronically gated tetramer binding CD8⁺ cells ($n \geq 5$). Gates for the memory precursor effector cells (MPEC) and short-lived effector cells (SLEC) are indicated. Data represent 3 separate experiments.



Supplemental Figure 5 Anti-CD8 treatment depletes memory CD8⁺ T cells. CD8⁺ T cells were depleted with anti-CD8 α antibody on days -3, -1, 1, 3, 5, and 17. Mice were infected with HKx31 virus on day 0 and treated daily with rapamycin or PBS. On day 27, a sample of blood from each mouse was analyzed for CD8 α expression and **(a)** H-2D^b PA₃₆₆ or **(b)** H-2D^b NP₃₄₄ tetramer binding. Data represent the average number of cells/ml of blood \pm s.e.m. of 15 mice per group and 3 independent experiments.



Supplemental Figure 6 Rapamycin does not increase the number or proportion of regulatory T cells. Mice were infected with HKx31 virus and received rapamycin or PBS daily. Spleens were harvested on the indicated days and analyzed for the (a) proportion and (b) number of CD4⁺ T cells that were Foxp3⁺. Data represent averages \pm s.e.m. of 5 mice per group and 2 independent experiments. (c-d) Blood was collected from mice treated with PBS or rapamycin 26 days after HKx31 infection and analyzed for the (c) proportion and (d) number of CD4⁺ Foxp3⁺ T cells per ml of blood. ($n = 18$ per group, **** $P < 0.0001$). Data are representative of 3 independent experiments. (e-f) Mice were infected with HKx31 virus, received rapamycin or PBS daily, and were challenged with Δ Vn1203. Five days following secondary infection, the indicated organs were harvested and analyzed for CD4⁺ Foxp3⁺ cells. Data represent averages \pm s.e.m. of 4 mice per group and 2 independent experiments.



Supplemental Figure 7 The number of GL7⁺ and BrdU⁺ cells is decreased in rapamycin-treated mice compared to controls. Mice were infected with HKx31virus and received rapamycin or PBS daily. Four and two hours prior to harvest, mice received an injection of BrdU. Spleen and MLN cells were harvested 15 or 20 days after HKx31 infection and stained with antibodies to B220, BrdU, and GL7 to determine (**a-b**) the number of GL7⁺ B cells (**c-d**) the number of BrdU⁺ B cells, and (**e-f**) the percent GL7⁺ B cells that are BrdU⁺. Data represent averages \pm s.e.m. ($n = 5$ per group; * $P < 0.05$ and ** $P < 0.01$, Mann-Whitney U test).

Supplemental Table I. Surface accessibility of differentially targeted epitopes

HA Strain	Differentially expressed in	Peptide sequence	Average 1.4Å SASA score	Average 4Å SASA score	Number of known antibody contact residues
Viet1203	PBS	EWSYIVEKANPVDLCYPGD	673.9	116.8	8
Viet1203	RAPA	FNDYEELKHLLSRINHFKEI	632.9	66.8	0
Viet1203	RAPA	RMEFFWTILKPNDAINFESN	554.0	85.0	3
Viet1203	PBS	NTKCQTPMGAINSSMPFHNI	751.4	166.6	8
Viet1203	PBS	DFHDSNVKNLYDKVRLQLRD	584.6	6.5	0
Viet1203	RAPA	SDQICIGYHANNSTEQVDTI	628.5	157.6	2
Viet1203	PBS	GNFIAPEYAYKIVKKGDSTI	587.8	117.7	0
Viet1203	PBS	DFHDSNVKNLYDKVRLQLRD	584.6	6.5	0
X31	PBS	PHRILDGIDCTLIDALLGDP	466.2	97.9	9
X31	PBS	RLNWLTKSGSTYPVLNVTMP	821.6	196.3	16
X31	PBS	KLATGMRNVPEKQTRGLFGA	844.5	212.0	8
X31	PBS	IAGFIENGWEGMIDGWYGFR	787.6	157.6	17
X31	PBS	RIQDLEKYVEDTKIDLWSYN	434.1	9.6	0
X31	PBS	HTIDLTDSEMKNLFKTRRQ	436.9	1.3	0
X31	PBS	FAISCFLLCVVLLGFIMWAC	n/a	n/a	n/a
X31	PBS	IMWACQQRGNIRCNICI	n/a	n/a	n/a
X31	PBS	IAGFIENGWEGMIDGWYGFR	787.6	157.6	17
X31	PBS	TDSEMKNLFKTRRQLRENA	454.0	1.51	0
X31	RAPA	IMWACQQRGNIRCNICI	n/a	n/a	0

Supplemental Table I Surface accessibility of differentially targeted epitopes. Antigens that were differentially targeted by one of the two experimental groups and their surface accessibility areas computed using a standard water-sized probe (1.4Å) and a larger 4Å probe to mimic accessibility to protein moieties. The last column lists the number of known antibody contact sites in the PDB database for each antigen.